Figure 1

Activation of the Transcription Factor NF-κB through TNF Receptor 2 in CT6 Cells

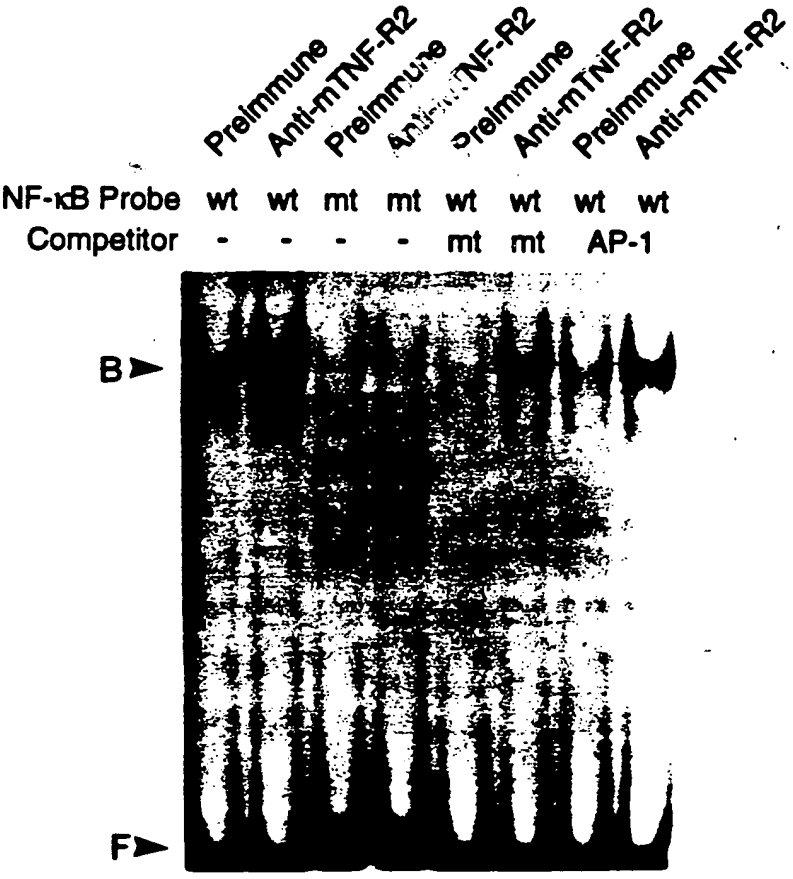

Figure 2

Immunoprecipitation of Human TNF Receptor 2

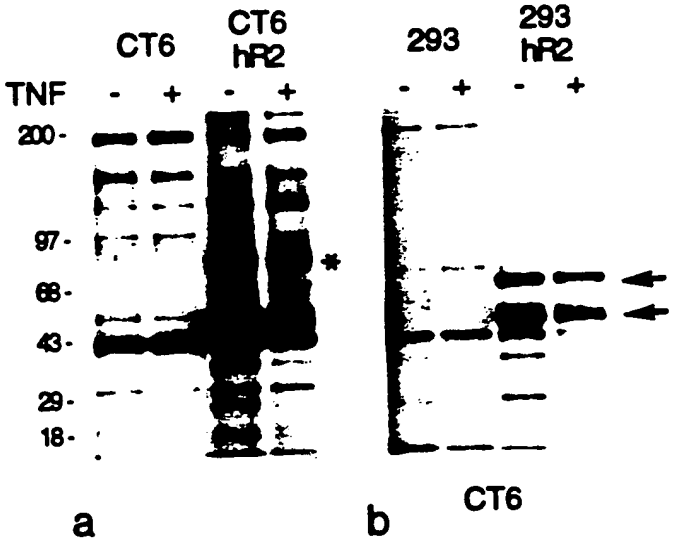

Figure 3

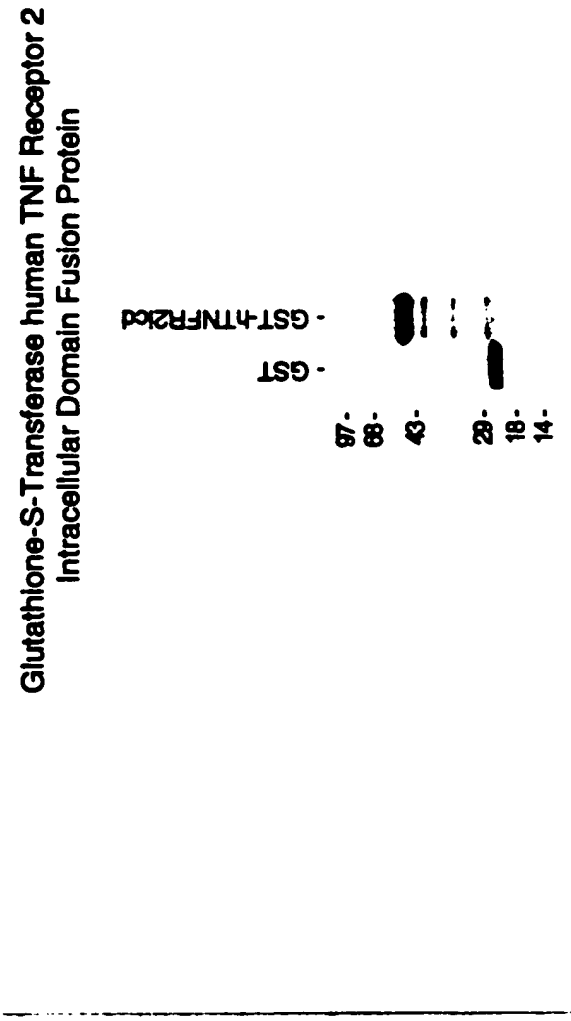
Glutathione-S-Transferase human TNF Receptor 2
Intracellular Domain Fusion Protein
GST-hTNFR2icd
GST
97-
68-
43-
29-
18-
14-

Figure 4

Coprecipitation of Glutathione-S-Transferase
Human TNF Receptor 2 Intracellular Domain
Fusion Protein in CT6 Cell Extracts

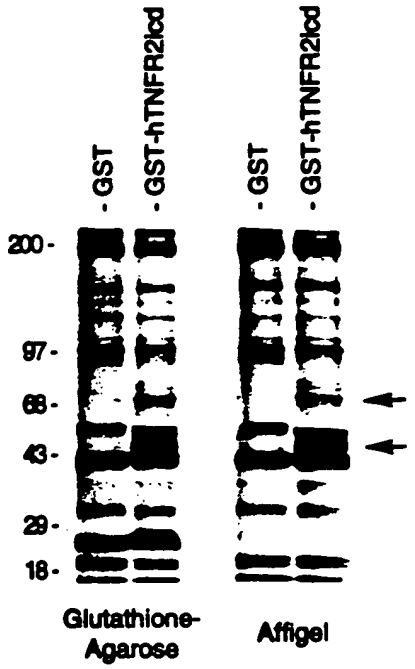

Figure 5

Coprecipitation of Glutathione-S-Transferase Mutant Human TNF Receptor 2 Intracellular Domain Fusion Proteins in CT6 Cell Extracts

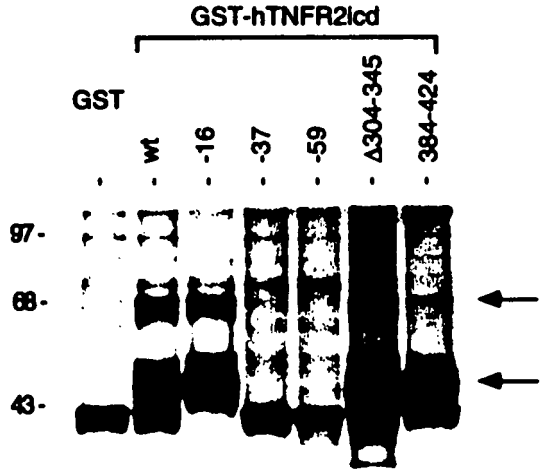

Figure 6

Competition of TNF Receptor 2 Associated Factors with Glutathione-S-Transferase TNF Receptor 2 Intracellular Domain Fusion Proteins

CT6

Figure 7

Coprecipitation of Glutathione-S-Transferase
Human TNF Receptor 2 Intracellular Domain
Fusion Protein in Jurkat Cell Extracts

Figure 8

Intracellular Localization of TNF Receptor 2 Associated Factors

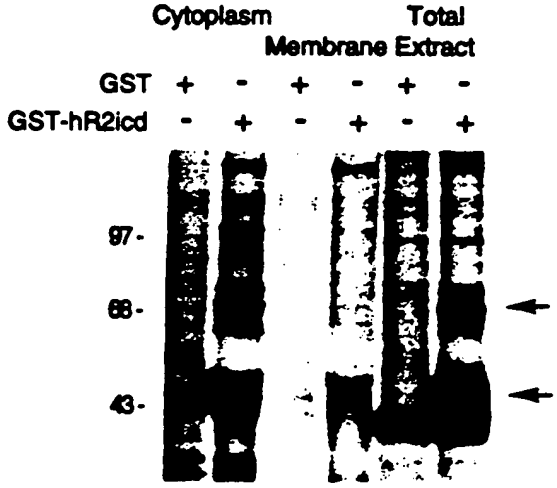

Figure 9

Purification of TNF Receptor 2 Associated Factors

```
   1  CCCAGCCCGGTTCTCTGCCCCAAGGACGCTACCGCCCAATGCGAGCAGAAGGCGGCGCACAGATACAGAAAGT
  74  GAGGCTCAGACATATTGAAGACCGTGTGACATAGGGTAGCCAAATGACAGTGTGAGAAAGTGACATTTACTCAAG
 149  GCCACCCAGATATCCTGGAGGACCCAGAACCCTGGAGATTCCCATCAGAAAGACCTTCTGGCCACCTGAAACCCC

   1    MetAlaSerSerSerAlaProAspGluAsnGluPheGlnPheGlyCysProProAlaProCysGlnAspPro
 224  AAGATGGCCTCCAGCTCAGCCCCTGATGAAAACGAGTTTCAATTTGGTTGCCCCCCCTGCTCCCTGCCAGGACCCA

  25  SerGluProArgValLeuCysCysThrAlaCysLeuSerGluAsnLeuArgAspAspGluAspArgIleCysPro
 299  TCGGAGCCCAGAGTTCTCTGCTGCACAGCCTGTCTCTCTGAGAACCTGAGAGATGATGAGGATCGGATCTGTCCT

  50  LysCysArgAlaAspAsnLeuHisProValSerProGlySerProLeuThrGlnGluLysValHisSerAspVal
 374  AAATGCAGAGCAGACAACCTCCATCCTGTGAGCCCAGGAAGCCCTCTGACTCAGGAGAAGGTTCACTCTGATGTA

  75  AlaGluAlaGluIleMetCysProPheAlaGlyValGlyCysSerPheLysGlySerProGlnSerMetGlnGlu
 449  GCTGAGGCTGAAATCATGTGCCCCTTTGCAGGTGTTGGCTGTTCCTTCAAGGGGAGCCCACAATCCATGCAGGAG

 100  HisGluAlaThrSerGlnSerSerHisLeuTyrLeuLeuLeuAlaValLeuLysGluTrpLysSerSerProGly
 524  CATGAGGCTACCTCCCAGTCCTCCCACCTGTACCTGCTGCTGGCGGTCTTAAAGGAGTGGAAATCCTCACCAGGC

 125  SerAsnLeuGlySerAlaProMetAlaLeuGluArgAsnLeuSerGluLeuGlnLeuGlnAlaAlaValGluAla
 599  TCCAACCTAGGGTCTGCACCCATGGCACTGGAGCGGAACCTGTCAGAGCTGCAGCTTCAGGCAGCTGTGGAAGCG

 150  ThrGlyAspLeuGluValAspCysTyrArgAlaProCysCysGluSerGlnGluGluLeuAlaLeuGlnHisLeu
 674  ACAGGGGACCTGGAGGTAGACTGCTACCGGGCACCTTGCTGTGAGAGCCAGGAAGAACTGGCCCTGCAGCACTTG

 175  ValLysGluLysLeuLeuAlaGlnLeuGluGluLysLeuArgValPheAlaAsnIleValAlaValLeuAsnLys
 749  GTGAAGGAGAAGCTGCTGGCTCAGCTGGAGGAGAAGCTGCGTGTGTTTGCAAACATTGTTGCTGTCCTCAACAAG

 200  GluValGluAlaSerHisLeuAlaLeuAlaAlaSerIleHisGlnSerGlnLeuAspArgGluHisLeuLeuSer
 824  GAAGTGGAGGCTTCCCACCTGGCACTGGCCGCCTCCATCCACCAGAGCCAGTTGGACCGAGAGCACCTCCTGAGC

 225  LeuGluGlnArgValValGluLeuGlnGlnThrLeuAlaGlnLysAspGlnValLeuGlyLysLeuGluHisSer
 899  TTGGAGCAGAGGGTGGTGGAATTACAGCAAACCCTGGCTCAAAAAGACCAGGTCCTGGGCAAGCTTGAGCACAGT

 250  LeuArgLeuMetGluGluAlaSerPheAspGlyThrPheLeuTrpLysIleThrAsnValThrLysArgCysHis
 974  CTGCGACTCATGGAGGAGGCATCCTTTGATGGTACTTTCCTGTGGAAGATCACCAATGTCACCAAGCGGTGCCAC

 275  GluSerValCysGlyArgThrValSerLeuPheSerProAlaPheTyrThrAlaLysTyrGlyTyrLysLeuCys
1049  GAGTCAGTGTGTGGCCGGACTGTCAGCCTCTTCTCTCCAGCTTTCTACACTGCCAAGTATGGTTACAAGTTGTGC

 300  LeuArgLeuTyrLeuAsnGlyAspGlySerGlyLysLysThrHisLeuSerLeuPheIleValIleMetArgGly
1124  CTGCGCTTGTACCTGAACGGGGATGGCTCAGGCAAGAAGACCCACCTGTCCCTCTTCATCGTGATCATGAGAGGA

 325  GluTyrAspAlaLeuLeuProTrpProPheArgAsnLysValThrPheMetLeuLeuAspGlnAsnAsnArgGlu
1199  GAATACGATGCTCTCCTGCCCTGGCCTTTCAGGAACAAGGTCACCTTTATGCTACTTGACCAGAACAACCGAGAG

 350  HisAlaIleAspAlaPheArgProAspLeuSerSerAlaSerPheGlnArgProGlnSerGluThrAsnValAla
1274  CATGCTATTGATGCCTTCCGGCCTGACCTGAGCTCAGCCTCCTTCCAGCGGCCACAGAGTGAGACCAACGTGGCC

 375  SerGlyCysProLeuPhePheProLeuSerLysLeuGlnSerProLysHisAlaTyrValLysAspAspThrMet
1349  AGCGGCTGCCCGCTCTTCTTCCCCCTCAGCAAGCTGCAGTCACCCAAGCACGCCTACGTCAAAGATGACACAATG

 400  PheLeuLysCysIleValAspThrSerAla
1424  TTCCTCAAATGCATTGTGGACACTAGTGCTTAGGGATGGGGGGAGGGGGTGTCTCCTGACAGAACCAGCTTAGAC
1499  TGGGGGACTTAGCTAGACAGCCAGGCCCTGCCTGCCCTTGGAGCCCACAGCCCACGACAAGGAGGAGCCAAGGCT
1574  GGCATGACTTCAGCGCCACAGCATGCTGGTTATGGCTGATGTGAGGCTGGAGAAACGTGTGCGTACAGAGACAGA
1649  GTGGAGGAGAAGACAGAAGTGCTCTTTTCACACAGACTACACGACACCAGGAGGCCAGCATGCCAGCAGCTTCTG
1724  AATGTTGAGACCAGCCTAGATCAGGATGAAAAGAGCCAGGCCTGAGGCTTGGACATTGAGCCAAGGCTATGGGGC
1799  CTAAGTGGAGGGGCACTCCTACCAGGACATTCTCTCGAGGTCAGGGCATAACTGGAAAAATGCCCCCATCTCTCT
1874  GTTCAGACTCAAAACTAGAACCACAGGGCAGAAGGGTCAGACATTAATGTGAATTTAACCTGCCCTGGACTGAGT
1949  TCCTATGTTAACAGACACGCAAACAGGTAAACCCAGAAACTGCCCTGGGAAATGCTTTCTGGCTGCATCTGGAGA
2024  TCTTTGATGTTTTTACCGACAAAACAAATAACAAAAGCCTTGAATTGCAAAAAAAAAAAAAAAAAA
```

Figure 10

08/779599

```
                                                              MetAlaAlaAlaSerValThrSerPro
   1
   1  GCGCGAAGACCGTTGGGGCTTTGTGGTGTGTGGGGGTTGTAACTCACATGGCTGCAGCCAGTGTGACTTCCCCT

  10  GlySerLeuGluLeuLeuGlnProGlyPheSerLysThrLeuLeuGlyThrArgLeuGluAlaLysTyrLeuCys
  75  GGCTCCCTAGAACTGCTACAGCCTGGCTTCTCCAAGACCCTCCTGGGGACCAGGTTAGAAGCCAAGTACCTCTGT

  35  SerAlaCysLysAsnIleLeuArgArgProPheGlnAlaGlnCysGlyHisArgTyrCysSerPheCysLeuThr
 150  TCAGCCTGCAAAAACATCCTGCGGAGGCCTTTCCAGGCCCAGTGTGGGCACCGCTACTGCTCCTTCTGCCTGACC

  60  SerIleLeuSerSerGlyProGlnAsnCysAlaAlaCysValTyrGluGlyLeuTyrGluGluGlyIleSerIle
 225  AGCATCCTCAGCTCTGGGCCCCAGAACTGTGCTGCCTGTGTCTATGAAGGCCTGTATGAAGAAGGCATTTCTATT

  85  LeuGluSerSerSerAlaPheProAspAsnAlaAlaArgArgGluValGluSerLeuProAlaValCysProAsn
 300  TTAGAGAGTAGTTCGGCCTTTCCAGATAACGCTGCCCGCAGAGAGGTGGAGAGCCTGCCAGCTGTCTGTCCCAAT

 110  AspGlyCysThrTrpLysGlyThrLeuLysGluTyrGluSerCysHisGluGlyLeuCysProPheLeuLeuThr
 375  GATGGATGCACTTGGAAGGGGACCTTGAAAGAATACGAGAGCTGCCACGAAGGACTTTGCCCATTCCTGCTGACG

 135  GluCysProAlaCysLysGlyLeuValArgLeuSerGluLysGluHisHisThrGluGlnGluCysProLysArg
 450  GAGTGTCCTGCATGTAAAGGCCTGGTCCGCCTCAGCGAGAAGGAGCACCACACTGAGCAGGAATGCCCCAAAAGG

 160  SerLeuSerCysGlnHisCysArgAlaProCysSerHisValAspLeuGluValHisTyrGluValCysProLys
 525  AGCCTGAGCTGCCAGCACTGCAGAGCACCCTGTAGCCACGTGGACCTGGAGGTACACTATGAGGTCTGCCCCAAG

 185  PheProLeuThrCysAspGlyCysGlyLysLysLysIleProArgGluThrPheGlnAspHisValArgAlaCys
 600  TTTCCCTTAACCTGTGATGGCTGTGGCAAGAAGAAGATCCCTCGGGAGACGTTTCAGGACCATGTTAGAGCATGC

 210  SerLysCysArgValLeuCysArgPheHisThrValGlyCysSerGluMetValGluThrGluAsnLeuGlnAsp
 675  AGCAAATGCCGGGTTCTCTGCAGATTCCACACCGTTGGCTGTTCAGAGATGGTGGAGACTGAGAACCTGCAGGAT

 235  HisGluLeuGlnArgLeuArgGluHisLeuAlaLeuLeuLeuSerSerPheLeuGluAlaGlnAlaSerProGly
 750  CATGAGCTGCAGCGGCTACGGGAACACCTAGCCCTACTGCTGAGCTCATTCTTGGAGGCCCAAGCCTCTCCAGGA

 260  ThrLeuAsnGlnValGlyProGluLeuLeuGlnArgCysGlnIleLeuGluGlnLysIleAlaThrPheGluAsn
 825  ACCTTGAACCAGGTGGGGCCAGAGCTACTCCAGCGGTGCCAGATTTTGGAGCAGAAGATAGCAACCTTTGAGAAC

 285  IleValCysValLeuAsnArgGluValGluArgValAlaValThrAlaGluAlaCysSerArgGlnHisArgLeu
 900  ATTGTCTGCGTCTTGAACCGTGAAGTAGAGAGGGTAGCAGTGACTGCAGAGGCTTGTAGCCGGCAGCACCGGCTA

 310  AspGlnAspLysIleGluAlaLeuSerAsnLysValGlnGlnLeuGluArgSerIleGlyLeuLysAspLeuAla
 975  GACCAGGACAAGATTGAGGCCCTGAGTAACAAGGTGCAACAGCTGGAGAGGAGCATCGGCCTCAAGGACCTGGCC

 335  MetAlaAspLeuGluGlnLysValSerGluLeuGluValSerThrTyrAspGlyValPheIleTrpLysIleSer
1050  ATGGCTGACCTGGAGCAGAAGGTCTCCGAGTTGGAAGTATCCACCTATGATGGGGTCTTCATCTGGAAGATCTCT

 360  AspPheThrArgLysArgGlnGluAlaValAlaGlyArgThrProAlaIlePheSerProAlaPheTyrThrSer
1125  GACTTCACCAGAAAGCGTCAGGAAGCCGTAGCTGGCCGGACACCAGCTATCTTCTCCCCAGCCTTCTACACAAGC

 385  ArgTyrGlyTyrLysMetCysLeuArgValTyrLeuAsnGlyAspGlyThrGlyArgGlyThrHisLeuSerLeu
1200  AGATATGGCTACAAGATGTGTCTACGAGTCTACTTGAATGGCGACGGCACTGGGCGGGGAACTCATCTGTCTCTC

 410  PhePheValValMetLysGlyProAsnAspAlaLeuLeuGlnTrpProPheAsnGlnLysValThrLeuMetLeu
1275  TTCTTCGTGGTGATGAAAGGCCCCAATGATGCTCTGTTGCAGTGGCCTTTTAATCAGAAGGTAACATTGATGTTG

 435  LeuAspHisAsnAsnArgGluHisValIleAspAlaPheArgProAspValThrSerSerSerPheGlnArgPro
1350  CTGGACCATAACAACCGGGAGCATGTGATCGACGCATTCAGGCCCGATGTAACCTCGTCCTCCTTCCAGAGGCCT

 460  ValSerAspMetAsnIleAlaSerGlyCysProLeuPheCysProValSerLysMetGluAlaLysAsnSerTyr
1425  GTCAGTGACATGAACATCGCCAGTGGCTGCCCCCTCTTCTGCCCTGTGTCCAAGATGGAGGCCAAGAATTCCTAT

 485  ValArgAspAspAlaIlePheIleLysAlaIleValAspLeuThrGlyLeu
1500  GTGCGGGATGATGCGATCTTCATCAAAGCTATTGTGGACCTAACAGGACTCTAGCCACCCCTGCTAAGAATAGCA
1575  GCTCAGTGAGGAGCTGTCACATTAGGCCAGCCAGGCCCTGCCACACACGGGTGGGCAGGCTTGGTGTAAATGCTG
1650  GGGAGGGCCTCAGCCTAGAGCCAATCACCATCACACAGAAAGGCAGGAAGAAGCCTCCAGTTGGCCTTCAGCTGG
1725  CAAACTGAGTTGGACGGTCCACTGAGCTCAAGGGCCTGGTGGAGCCCGCTGGGGAGCTTCTCAGCTTTCCAATAG
1800  GAAAGCTCCTGCTGTCTCCTCTGTCTGGGGAAGGGAGAGACCTGTAGGTGGGTGCTCAGAAAGGGCCTCTCCAGA
1875  GAGAGTCTCAAGAGCTGCAGCAGGAGCAAAGTGACTGGCCTTCCCCACCCCATCCTTTGGAAAAGAGGTAGCGGC
1950  TACACAGGAGAAGGCATGCGCCTGCAGGGTGTAGCCCAAGAGAGAAGCTCTCTGAGACATAGGCCCTCACTGGAG
2025  AAGGGCCTGCCTGGGCTGCACAGCCTTGCCAGGTGGCCTGTATGGGGGAGAAGTGATTAAATGTTGAGATGTCAC
2100  ACGACAAAAAAAAAAAAAAAAA
```

Figure 11

A

Figure 12a

TRAF2	(mouse)	31	KYLCSACKNILRRPFQA QCGHRYCSFCLTSI LSS GPQNCAACVYE
COP1	(*A. thaliana*)	49	DLLCPICMQIIKDAFLT ACGHSFCYMCIITH LRN KSDCPCCSQH
EFP	(human)	10	ELSCSICLEPFKEPVTT PCGHNFCGSCLNETWA VQG SPYLCPQCRAV
RAD-18	(*S. cerevisiae*)	25	LLRCHICKDFLKVPVLT PCGHTFCSLCIRTH LNN QPNCPLCLFE
UVS-2	(*N. crassa*)	31	AFRCHVCKDFYDSPMLT SCNHTFCSLCIRRC LSV DSK CPLCRAT
RAG-1	(human)	290	SISCQICEHILADPVET NCKHVFCRVCILRC LKV MGSYCPSCRYP
SS-A/Ro	(human)	13	EVTCPICLDPFVEPVSI ECGHSFCQECISQV GKG GGSVCAVCRQR
RING1	(human)	16	ELMCPICLDMLKNTMTTKECLHRFCSDCIVTA LRS GNKECPTCRKK
RPT-1	(mouse)	12	EVTCPICLELLKEPVSA DCNHSFCRACITLNYESNRNTDGKGNCPVCRVP
RFP	(human)	13	ETTCPVCLQYFAEPMML DCGHNICCACLARCWGTA ETNVSCPQCRET
c-cbl	(human)	378	FQLCKICAENDKDVKIE PCGHLMCTSCLTS WQESEGQ GSSGCPFCRCE
			X11-12 X10-16
consensus			---C--C----------C-H--C--C----------C--C---

B

Figure 12b

TRAF2	(mouse)	157	CPKRSLSCQHC RAPCSHVDLEVHYE VC
		182	PKFPLTCDGCGKKKIPRETFQDHVR AC
DG17	(*D. discoideum*)	171	GGFKLVTCDFCKRDDIKKKELETHYK TC
TFIIIA	(*X. laevis*)	189	QD LAVCDVCNRKFRHKDYLRDHQK TH
XLCOF14	(*X. laevis*)	1	TGKYPFICSECGKSFMDKRYLKIHSN VH
XFIN	(*X. laevis*)	1225	TGEKPYTCTVCGKKFIDRSSVVKHSR TH
ZFY1/2	(mouse)	521	RKKFPHICGECGKGFRHPSALKKHIR VH
MFG2	(mouse)	293	SEEKPFECEECGKKFRTARHLVKHQR IH
RAD18	(*S. cerevisiae*)	183	PNEQMAQCPICQQFYPLKALEKTHLD EC
UVS-2	(*N. crassa*)	182	PDDGLVACPICLTRM KEQQVDRHLDTSC

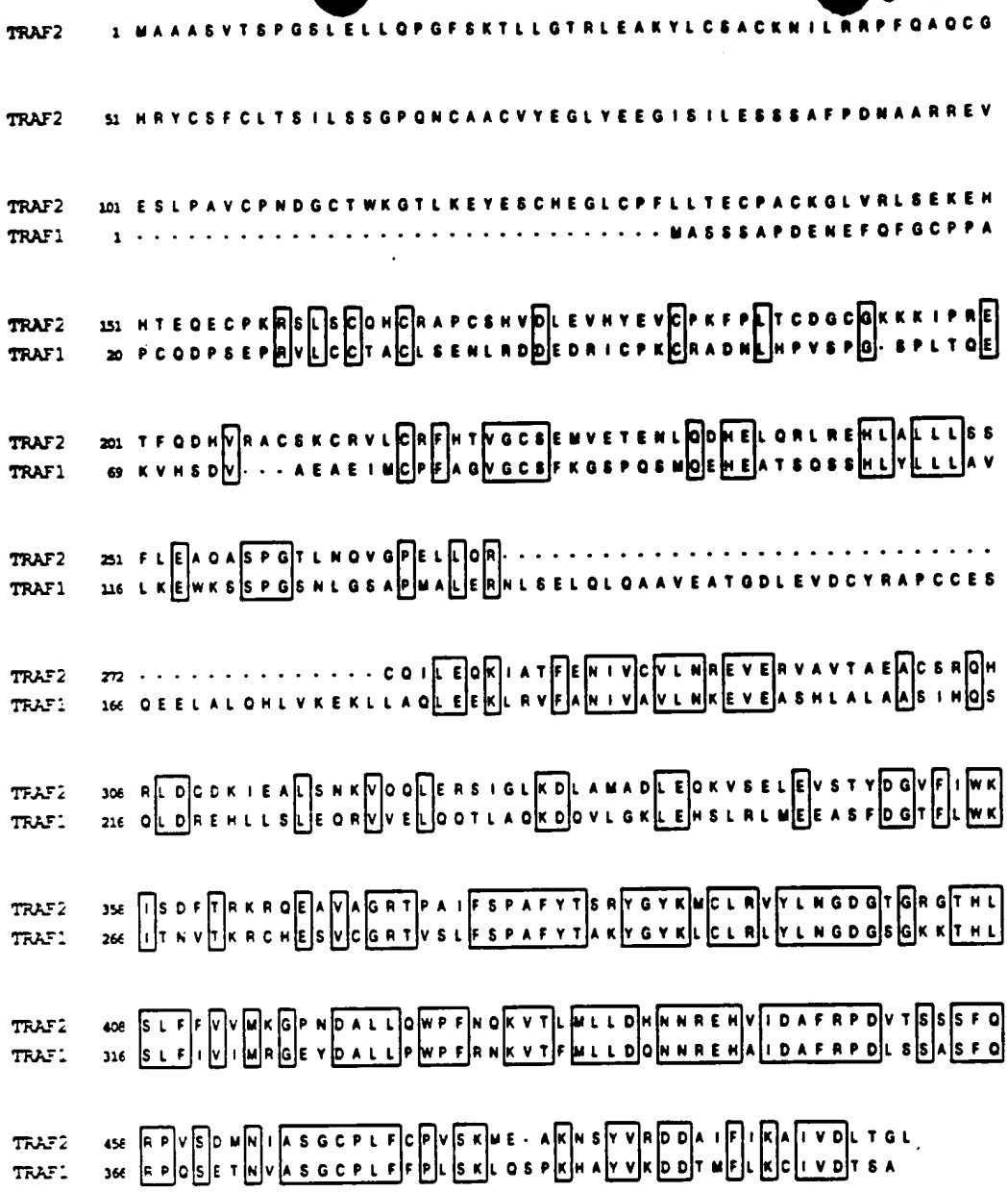

Figure 13

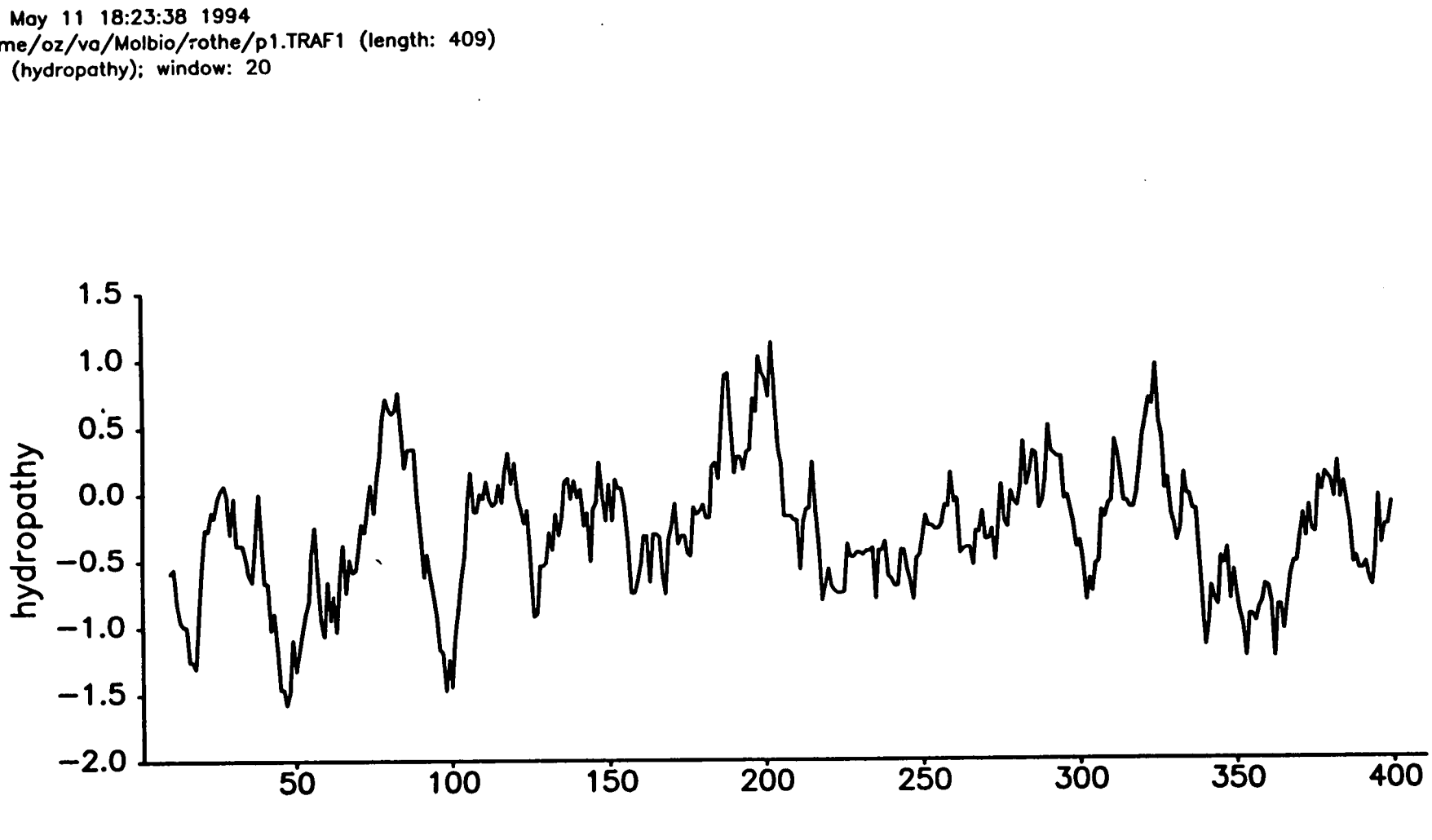

Figure 14a

wed May 11 18:23:52 1994
/home/oz/va/Molbio/rothe/p1.TRAF2 (length: 501)
kyte (hydropathy); window: 20

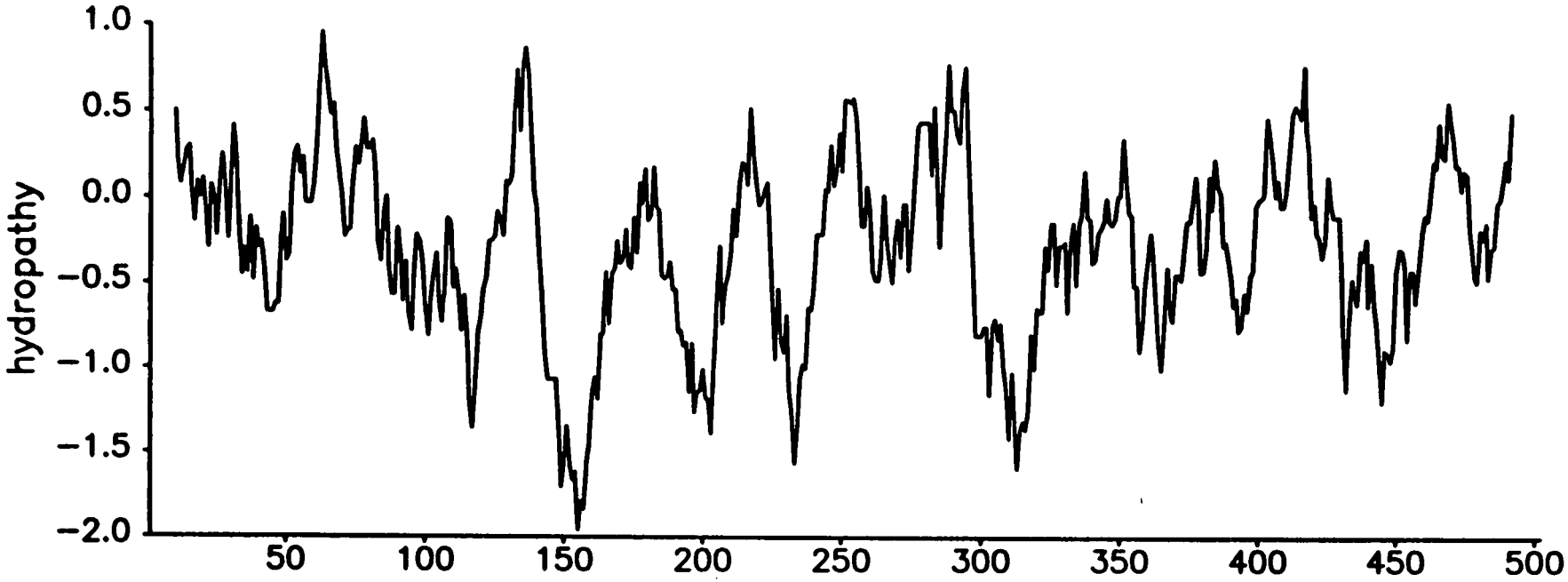

Figure 14b

TRAF Expression in CT6 Cells

Figure 15a

Figure 15b

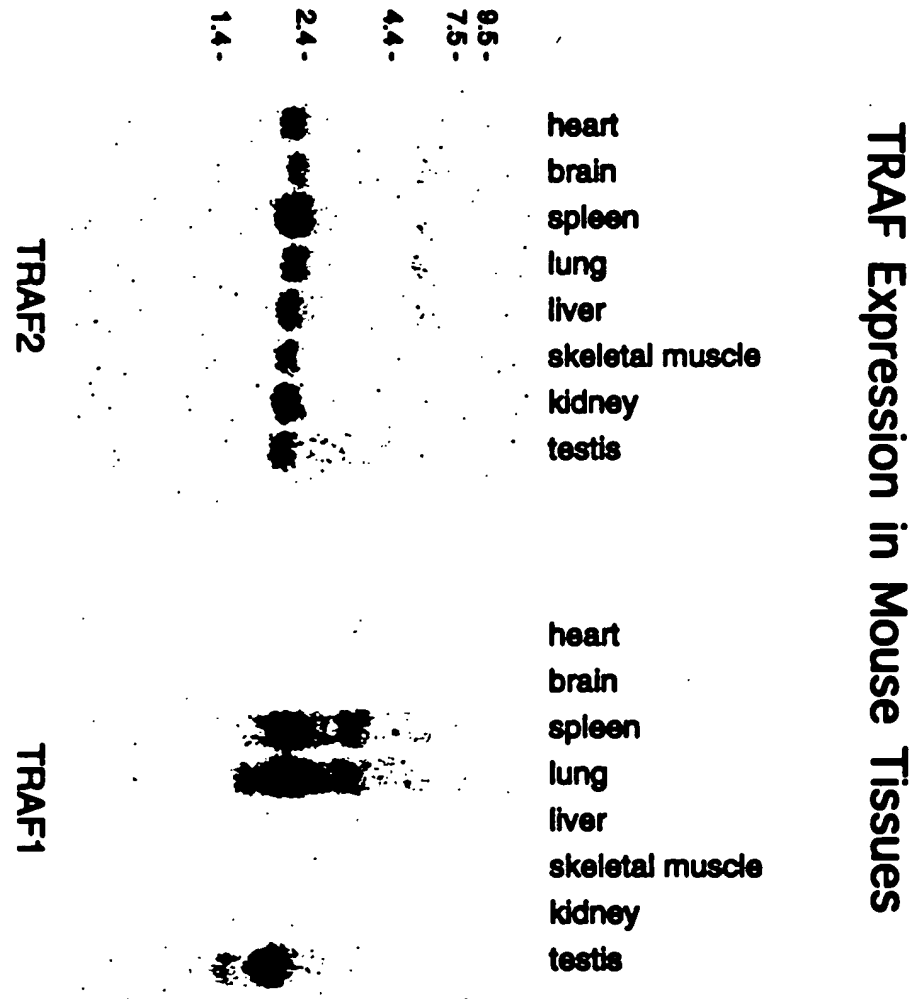
TRAF Expression in Mouse Tissues
TRAF2
TRAF1
1.4-
2.4-
4.4-
7.5-
9.5-
heart
brain
spleen
lung
liver
skeletal muscle
kidney
testis
heart
brain
spleen
lung
liver
skeletal muscle
kidney
testis

Figure 16

A Glutathione-S-Transferase TRAF2 Fusion Protein Coprecipitates the Human TNF-R2 in 293 Cell Extracts